VARIATIONS OF COLOR PATTERN IN HYBRIDS OF THE GOLDFISH, CARASSIUS AURATUS

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This paper gives an account not only of the differences between fish arising from the same genetic cross but also of the variations of color pattern taking place during the life of individual fish.

The cross between the common goldfish and the transparent shubunkin which are both varieties of the species *Carassius auratus* was first subjected to genetic analysis by Berndt (1925 and 1928) and Chen (1925 and 1928). The results indicated that the two parental types are genetically distinguishable by a single gene difference. The formulae as denoted by Chen are: common goldfish TT, the transparent shubunkin $T'T'$, and the hybrid TT'. This hybrid is known to the fanciers as the calico shubunkin. The common goldfish, which is quite brown or black during youth, changes to the familiar orange or red type by destruction of part or nearly all of its melanophores (Berndt, 1925; Goodrich and Hansen, 1931). This type also carries at least two layers of reflecting tissue, one beneath the scale layer and the other backing each individual scale. The transparent shubunkin has lost most of the chromatophores (both melanophores and xanthophores) and also most of the reflecting tissue. The heterozygous type, or calico fish, shows great variability in the distribution of both melanophores and xanthophores and there is no bilateral symmetry of pattern. A deep abdominal layer of reflecting tissue is present and a few scales are also backed with the tissue. For full details, papers by Chen (1928) or Goodrich and Hansen (1931) may be consulted.

Goodrich and Hansen (1931) made a detailed comparative study of the history of the melanophores of the three phenotypes covering the first eight weeks after hatching during which period the fish grew from 4.5 mm. to about 33 mm. in length. It was found that the history of the three types was similar for the first week (to 9 mm.) showing a uniform rate of multiplication of the chromatophores. After this the three types diverged. The normal goldfish showed a very rapid

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and uniform increase in number of chromatophores. In the transparent shubunkin the melanophores began to disintegrate until nearly all were destroyed. The hybrid, however, was found to be highly variable, showing great diversity between individuals. New cells appeared; others were destroyed. It gave the impression of a conflict between the cell proliferation and cell destruction.

Material and Methods

This paper continues the observations on the melanophore pattern of the heterozygous type beginning where the previous study was discontinued. The work was begun during the summer of 1937 with fish varying from 23 to 36 mm. in length (tip of mouth to base of caudal fin). The fish were chiefly obtained from the Grassyfork Fisheries of Martinsville, Indiana, to which institution we are greatly indebted. The hybrid fish were obtained directly from the hatchery which raises them regularly for the market. Records were made by photographing one side of the fish at intervals of approximately one month, but the periods were lengthened to longer intervals during the last six months. Ten of the fish are still under observation at this time, one year and six months after the start of the work. They vary from 47 to 58 mm. in length. All others that were started died. Anaesthetization, necessary for photography, proves to be fatal in some cases.

The individuals differ markedly from each other. For purposes of description two types, *A* and *B*, may be recognized, but it should be understood that there are intermediate gradations. Type *A* shows a relatively uniform distribution of melanophores on the dorsal half of the body and extending variably below the lateral line (Figs. 1 and 2). In Type *B*, the distribution of melanophores is much more uneven. They tend to be aggregated in clusters (Figs. 4 and 5). Xanthophores are present in both types and are unevenly distributed, but are not studied in this paper as it is very difficult to distinguish and identify the individual cells.

History of Color Patterns

Type A

It is possible with these fish to enumerate and reidentify from time to time all cells of large areas on the photographed side of the body. Except in the cases where wholesale destruction of melanophores occurs, it is found that few cells are lost and that individual cells have long life. An example may be taken (our fish number *MG-3*) on which 907 cells were enumerated and located on the side of the body (see

Table I for this and other references to cell counts). The first photograph was taken August 2, 1937 and the last February 17, 1939 making a total series of 18 photographs. During this time, 50 of the 907 cells disappeared and three new ones appeared. Figure 1 is the photograph

TABLE I

This table gives records of photographs of four of the fish studied. The dates are accurate only for *MG-3* as it was not always possible to take all photographs on the same day.

	MG-3 Calico Type *A* 907 cells		*MG-4* Calico Type *B* 97 cells, 2 cl.		*MG-15* Transparent 21 cells		*MG-16* Calico Type *A* 613 cells	
	D	*A*	*D*	*A*	*D*	*A*	*D*	*A*
July 17, 1937	0	0	0	0(4)				
Aug. 2	0	0(1)	9	1 cl	0	0	0	0
Aug. 26	1	0	6	2 1 cl	0	0	6	0
Sept. 20	5	0	2	0	0	0	1	0
Oct. 18	5	0	0	0	1	0	0	1 cl
Nov. 16	5	0	7	0	1	0	(6)	0
Dec. 15	4	0	4	1 cl	0	0		0
Jan. 26, 1938	2	0	3	4 cl	0	0		0
Feb. 23	4	0	7	2 cl	0	0		0
Mar. 28	1	0	1	3 cl	0	0		0
May 2	4	1	1	0	1	0		0
June 7	9	1	1	0	1	0	(7)	0
July 16	2	1	0	0	0	0		0
Aug. 5	2	0	1	0	0	0		0
Sept. 13	–	–	8	0	0	0		0
Oct. 12	4	0	1	0	0	0		0
Nov. 14	0	0	0	0	0	0		0
Jan. 4, 1939	0	0(2)	0	0(5)	0	0		0
Feb. 17	2	0	0	0	0	0		0
Totals	50	3	51	2 12 cl	4	0	All	1 cl

D—number of cells that disappeared since preceding photograph.
A—number of new cells appearing since preceding photograph.
cl—cell cluster or spot.
(1), (2), (4), (5) indicate pictures reproduced in Figs. 1, 2, 4, and 5.
(6)—time of beginning of wholesale destruction of melanophores.
(7)—time at which all melanophores were destroyed.

taken August 2, 1937 and Fig. 2 that of January 4, 1939. The dotted lines outline arbitrarily delimited areas marked on the prints to facilitate the counting and identification of cells. The small circles indicate the former location of cells that have disappeared. Figure 2 is taken at a lower magnification than Fig. 1 and fish had grown from 26 mm.

to 58 mm. in length (exclusive of caudal fin). Figure 3, however, shows the rectangular area of Fig. 2 raised to the same magnification as Fig. 1.

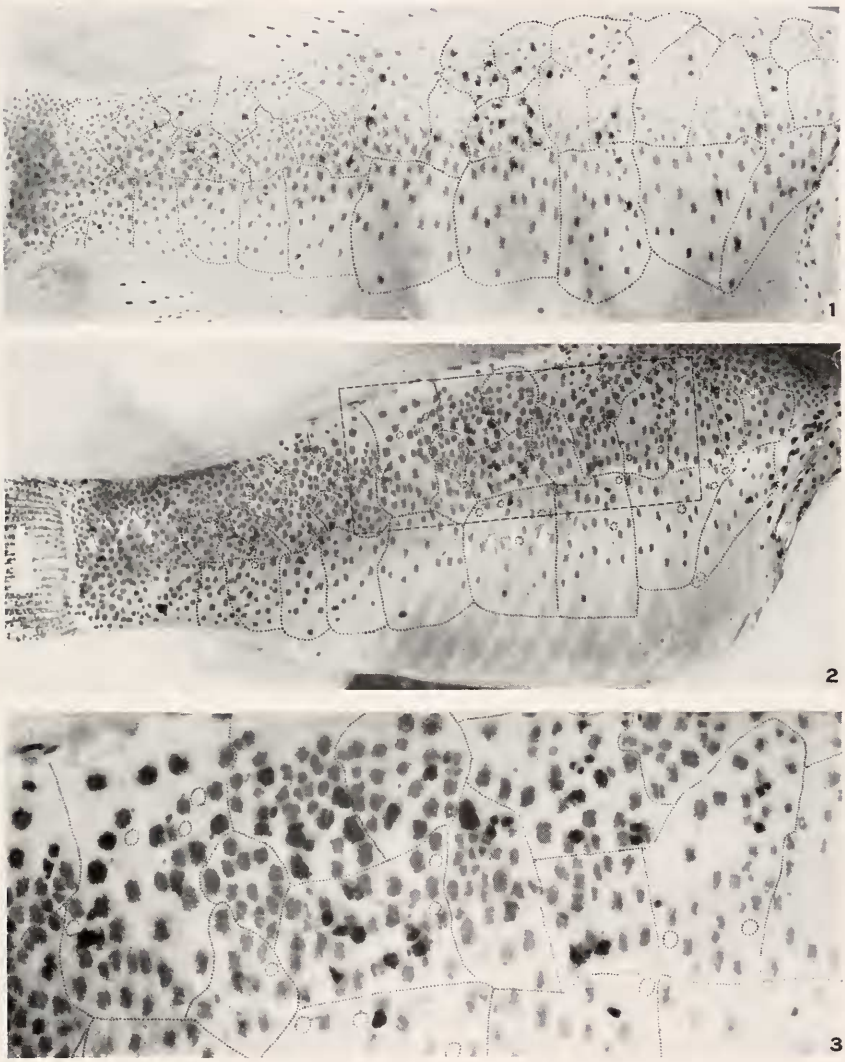

FIG. 1. Fish *MG*-3. Photograph taken August 2, 1937. × 5½. This is a "Type A" calico shubunkin; 907 cells are located in outlined areas.

FIG. 2. Fish *MG*-3. Photograph taken January 4, 1939. × 2½. Fifty-two cells have been lost and 3 new cells appeared since record of Fig. 1. Dotted circles indicate location of cells that have disappeared.

FIG. 3. Section outlined by dashes in Fig. 2 enlarged to same magnification as in Fig. 1, showing increase in size of area and of cells.

Type B

These fish show the irregular mottling which is prized by the fanciers. The dark spots are usually clusters of small melanophores too densely crowded to count. Of 97 selected on the first photograph of *MG*-4 on July 18, 1937, 46 remained on January 1, 1939 (Figs. 4 and 5). In the meanwhile, however, others have appeared and

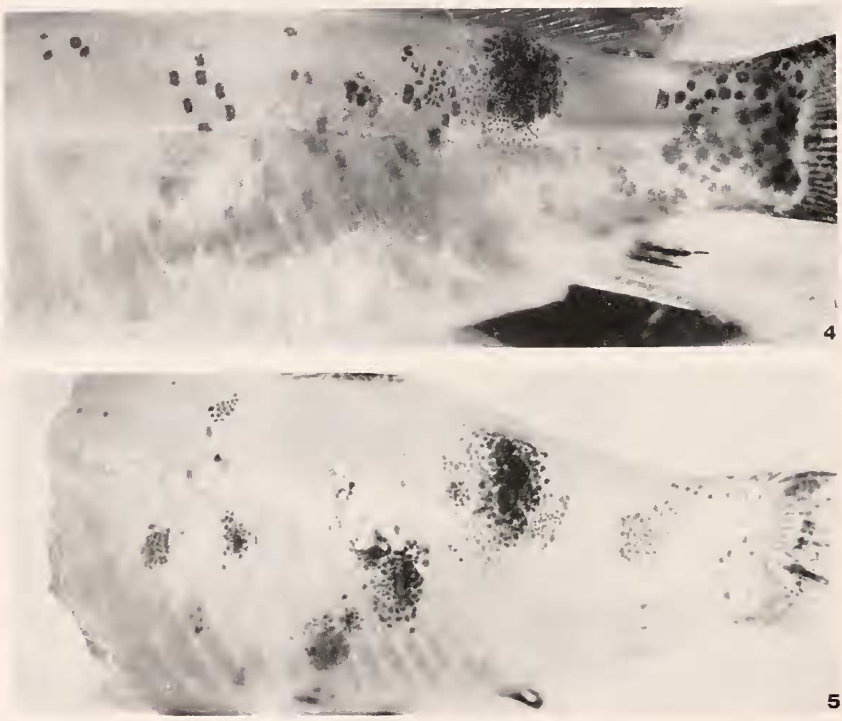

FIG. 4. Fish *MG*-4. Photograph taken July 18, 1937. × 6½.
FIG. 5. *MG*-4. Photograph taken January 4, 1939. × 2½. Fifty-one cells disappeared and 12 new cell clusters appeared in area under observation.

there has been a notable eruption of spots, or clusters of melanophores—12 altogether on the left side. These spots are first recognized as one or a few minute melanophores which rapidly increase in number. A spot for a time is often bounded by the posterior edge of a scale.

Indeterminate Types

In many cases the clusters of small cells appear among, or superficial to, cells uniformly distributed and in this way combine character-

istics of Types *A* and *B*. An example is *MG*-5, where it was possible to identify cells only in a small area. Ten disappeared out of 164 in this area, but six new clusters of cells have arisen similar to those discussed under Type *B*.

Extensive Cell Destruction

Occasionally a sweeping destruction of melanophores occurs within a few weeks. This is similar to the process in the ordinary goldfish, which is gold because melanophores but not xanthophores have been destroyed. This change most frequently takes place in ordinary goldfish at about three months of age but may occur much later (Cf. Berndt, 1925; Chen, 1925; and Goodrich and Hansen, 1931). It occurred in two of the calico shubunkins which we had under observation in this series. The history of one of these, *MG*-16, is given in Table I and in this the breakdown occurred at about eight months of age.

Increase in Size of Cells

As mentioned above, Fig. 3 shows the rectangular area marked on Fig. 2 enlarged to the same magnification as Fig. 1. The comparison of Figs. 1 and 3 then shows the actual increase in size of the area outlined. It also shows that the individual cells, which for the most part show approximately the same degree of melanin dispersion in both pictures, have definitely increased in size. It, therefore, appears that, in so far as the melanophores are concerned, the increase of body size has involved an enlargement of cells rather than a multiplication of cells.

Discussion

These observations not only show that there is much variation among individuals of these hybrids but also that each individual is variable in respect to color patterns displayed during its life cycle. The heterozygous type, as noted for earlier developmental stage by Goodrich and Hansen (1931), continues in later stages to be in a condition of unstable equilibrium between opposing tendencies—those of cell multiplication and cell destruction.

Fukui (1927 and 1930) has shown that the destruction of melanophores in the ordinary goldfish tends to take place in definitely bounded areas, giving rise to some degree of uniformity of pattern in black- and goldfish. These areas, he believes, correspond to regions of looser subcutaneous tissue bounded by more dense tissue. In effect, these may be perhaps regarded as sinuses filled with tissue fluids or lymph.

His experiments with injection of adrenalin showed a restoration of pigment which tended to be circumscribed in such areas. These results suggest that endocrine factors operating on such a region bring about under certain conditions the destruction of chromatophores and under other conditions the production of pigment. Fukui suggests that pigment destruction is due to a higher metabolic rate in these areas, but this might be stimulated by the chemical environment.

In contrast to the above, the origin of new spots or cell clusters is entirely irregular, having no relation to the areas described by Fukui. It, therefore, seems unlikely that their location can be due to endocrinal conditions.

It then seems probable that the goldfish presents a new example of the dual gene control such as has been suggested in the plumage of birds. In the case here described the direct gene action may control cell multiplication, resulting in the formation of cell clusters or spots, while remote gene control of "endocrinal regulation" may cause the destruction of cells (see Danforth, 1932, p. 33).

A discussion of the developmental origin of cell clusters will be presented in the companion paper, Goodrich and Trinkaus (p. 188).

Summary

1. The F_1 heterozygous types from the cross of the common goldfish with the transparent shubunkin (both of the species *Carassius auratus*) show not only a great range of variability between individuals, but frequently the pattern of a single individual changes markedly during the life cycle. This is due to destruction and emergence of chromatophores producing a varying pattern. It is suggested that the multiplication of cells is an example of "direct gene control" and the destruction is due to "endocrinal regulation" or remote gene control.

2. Many individual melanophores are long-lived, having been identified at the beginning and end of the 19-month period of observation.

3. Such long-lived melanophores gradually increase in size during the growth of the fish.

BIBLIOGRAPHY

Berndt, Wilhelm, 1925. Vererbungstudien an Goldfischrassen. *Zeitschr. f. Indukt. Abst. u. Vererb.*, **36**: 161–349.

Berndt, Wilhelm, 1928. Wildform und Zierrassen bei der Karausche. *Zool. Jahrb., Abt. Allgem. Zool. u. Physiol.*, **45**: 841–972.

Chen, Shisan C., 1925. Variation in the external characters of goldfish, Carassius auratus. *Contr. Biol. Lab. Sci. Soc. China.*, **1**: 1–65.

Chen, Shisan C., 1928. Transparency and mottling, a case of mendelian inheritance in the goldfish, Carassius auratus. *Genetics*, **13**: 434–452.

Danforth, C. H., 1932. In Allen, E. Sex and Internal Secretions, pp. 12–54. Baltimore.

FUKUI, KEN'ICHI, 1927. On the color pattern produced by various agents in the goldfish. *Folia Anat. Japon.*, 5: 257–302.

FUKUI, KEN'ICHI, 1930. The definite localization of the color pattern in the goldfish. *Folia Anat. Japon.*, 8: 283–312.

GOODRICH, H. B., AND I. B. HANSEN, 1931. The postembryonic development of mendelian characters in the goldfish, Carassius auratus. *Jour. Exper. Zool.*, 59: 337–358.

GOODRICH, H. B., AND J. P. TRINKAUS, 1939. The differential effect of radiations on mendelian phenotypes of the goldfish, Carassius auratus. *Biol. Bull.*, 77: 188–195.